Serial Number: 10/081,170 Filing Date: February 22, 2002

Title: METHODS TO IDENTIFY MUTANT CELLS WITH ALTERED SIALIC ACID

Page 2 Dkt: 800.029US1

## **IN THE CLAIMS**

Please amend the claims as follows.

- 1. (Currently Amended) An isolated mutant mammalian or avian cell comprising decreased levels of N-acetylneuraminic acid and/or decreased levels of N-glycolylneuraminic acid relative to a corresponding wild-type cell which wild-type cell supports efficient influenza virus replication, wherein the mutant cell is selected for resistance to growth inhibition by a lectin which binds terminal sialic acid containing residues in sialic acid-containing host cell receptor, wherein the decreased levels of N-acetylneuraminic acid and/or decreased levels of N-acetylneuraminic acid and/or decreased levels of N-acetylneuraminic acid and/or decreased levels of N-glycolylneuraminic acid are in the absence of exogenous sialidase.
- 2. (Canceled)
- 3. (Previously Presented) The isolated mutant cell of claim 1 which is a swine, bovine, simian or canine cell.
- 4. (Previously Presented) The isolated mutant cell of claim 1 wherein the wild-type cell is a Madin-Darby canine kidney (MDCK) cell.
- 5. (Previously Presented) The isolated mutant cell of claim 1 which is a mink cell.
- 6. (Original) The isolated mutant cell of claim 5 which is a mink lung cell.
- 7. (Canceled)
- 8. (Original) The isolated mutant cell of claim 1 which has decreased levels of *N*-acetylneuraminic acid.

Serial Number: 10/081,170 Filing Date: February 22, 2002

Title: METHODS TO IDENTIFY MUTANT CELLS WITH ALTERED SIALIC ACID

Page 3 Dkt: 800.029US1

9. (Original) The isolated mutant cell of claim 1 which has decreased levels of *N*-glycolylneuraminic acid.

- 10. (Original) The isolated mutant cell of claim 1 which has decreased levels of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid.
- 11. (Original) The isolated mutant cell of claim 1 which has at least ten fold lower levels of *N*-acetylneuraminic acid and at least 2 fold lower levels of *N*-glycolylneuraminic acid relative to the corresponding wild-type cell.
- 12. (Withdrawn-Currently Amended) A method to isolate a cell that has decreased levels of receptors for influenza virus, comprising:
  - a) contacting a population of cells permissive for influenza virus replication and sensitive to lectin growth inhibition with an amount of lectin so as to yield cells that are resistant to growth inhibition by the lectin, wherein the lectin specifically binds terminal sialic acid containing residues in sialic acid containing cell receptors; and
  - b) isolating a lectin-resistant cell having decreased levels of receptors for influenza virus wherein the lectin-resistant cell has decreased levels of *N*-acetylneuraminic acid and/or decreased levels of *N*-glycolylneuraminic acid, and wherein the decreased levels of *N*-acetylneuraminic acid and/or decreased levels of *N*-glycolylneuraminic are an inherited phenotype.
- 13. (Withdrawn) The method of claim 12 wherein the lectin is *Maakia amurensis* lectin.
- 14. (Withdrawn) The method of claim 12 wherein the lectin is Sambucus nigra lectin.
- 15. (Canceled)
- 16. (Withdrawn) The method of claim 12 wherein the lectin is *Tritrichomonas mobilensis* lectin.

Serial Number: 10/081,170

Filing Date: February 22, 2002

Title:

METHODS TO IDENTIFY MUTANT CELLS WITH ALTERED SIALIC ACID

Page 4 Dkt: 800.029US1

17. (Withdrawn) The method of claim 12 wherein the lectin specifically binds sialic acid linked to galactose by  $\alpha(2-3)$  or  $\alpha(2-6)$  linkages.

- 18. (Withdrawn) The method of claim 12 wherein the lectin specifically bind siglic acid linked to N-acetylgalactosamine by  $\alpha(2-6)$  linkages.
- 19. (Withdrawn) The method of claim 12 wherein the cells are mammalian cells.
- 20. (Withdrawn) The method of claim 19 where the cells are canine, swine, bovine, mink, human or simian cells.
- 21. (Withdrawn) The method of claim 12 wherein the population of cells is a population of MDCK cells.
- 22. (Withdrawn) A resistant cell isolated by the method of claim 12.
- 23. (Withdrawn) The cell of claim 22 which is resistant to growth inhibition by Maakia amurensis lectin or Sambucus nigra lectin.
- 24. (Withdrawn) The cell of claim 22 which is resistant to growth inhibition by Maakia amurensis lectin and Sambucus nigra lectin.
- 25. (Withdrawn) A method of propagating influenza viruses having reduced sialidase activity, comprising: contacting the mutant cell of claim 1 or the resistant cell of claim 22 with an amount of an influenza virus having reduced sialidase activity so as to yield progeny virus.
- 26. (Withdrawn) Progeny virus obtained by the method of claim 25.

Serial Number: 10/081,170

Filing Date: February 22, 2002

Title: METHODS TO IDENTIFY MUTANT CELLS WITH ALTERED SIALIC ACID

Dkt: 800.029US1

Page 5

27. (Withdrawn) A method of using a host cell having decreased levels of sialic acid

containing host cell receptors for influenza virus, comprising:

a) contacting the isolated mutant cell of claim 1 or the resistant cell of claim 22 with an

amount of an influenza virus having substantially wild-type levels of sialidase activity so as

to yield progeny virus; and

b) serially propagating the progeny virus with the mutant cell of claim 1 or the resistant

cell of claim 22 so as to yield adapted viruses which efficiently replicate in the mutant

cell, the lectin-resistant cell or the agglutinin-resistant cell.

28. (Withdrawn) The method of claim 27 further comprising isolating the adapted virus.

29. (Withdrawn) Isolated adapted virus obtained by the method of claim 27 which adapted

virus does not have a mutation in the HA gene relative to the virus having substantially wild-type

levels of sialidase activity.

30. (Withdrawn) The method of claim 25 or 27 wherein the influenza virus is type A

influenza virus.

31. (Withdrawn) The method of claim 25 or 27 wherein the influenza virus is type B

influenza virus.

32. (Previously Presented) The isolated mutant cell of claim 1 which is resistant to Maakia

amurensis lectin and Sambucus nigra lectin.

33. (Previously Presented) The isolated mutant cell of claim 1 in which influenza virus with

reduced sialidase activity efficiently replicates.

34. (Previously Presented) The isolated mutant cell of claim 1 wherein the lectin specifically

binds sialic acid linked to galactose by  $\alpha(2-3)$  or  $\alpha(2-6)$  linkages.

Serial Number: 10/081,170 Filing Date: February 22, 2002

Title: METHODS TO IDENTIFY MUTANT CELLS WITH ALTERED SIALIC ACID

Page 6 Dkt: 800.029US1

35. (Previously Presented) The isolated mutant cell of claim 1 wherein the lectin specifically binds sialic acid linked to N-acetylgalactosamine by  $\alpha(2-6)$  linkages.

36. (Currently Amended) An isolated mutant mammalian or avian cell emprising which in the absence of exogenous sialidase comprises decreased levels of terminal sialic acid-containing host cell receptors for influenza virus relative to a corresponding wild-type cell which wild-type cell supports efficient influenza virus replication, wherein the mutant cell is resistant to growth inhibition by *Maakia amurensis* lectin and/or *Sambucus nigra* lectin.